Zwitterionic Hydrophilic Interaction Liquid Chromatography (HILIC) for Ion Analysis

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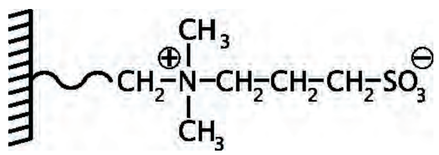
INTRODUCTION

Inorganic and organic ions are hydrophilic compounds. They may be analyzed by ion chromatography (IC) or ion-pair reversed-phase liquid chromatography (IP-RPLC). However, typical drawbacks are low detection sensitivity and poor compatibility with contemporary detection methods, e.g. mass spectrometry (MS), evaporative light scattering detector (ELSD) and Corona detector, due to the high levels of salts or additives required in the mobile phase.

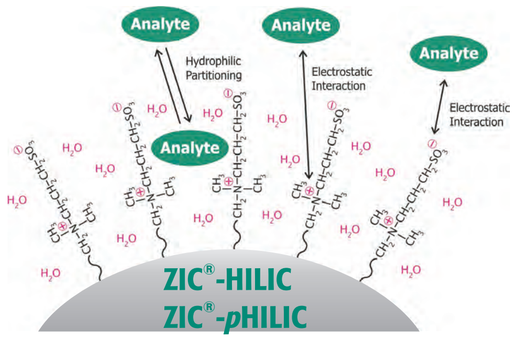
HILIC (hydrophilic interaction liquid chromatography)[1] is a technique that can address these problems since a high content of volatile organic solvent and buffers are used for separation. MS and ELSD detection can thus be greatly enhanced.

Both charged and neutral hydrophilic compounds are possible to be retained in HILIC. The zwitterionic stationary phase ZIC®-HILIC possesses permanent zwitterionic functional groups. Its zwitterionic properties are maintained regardless of mobile phase pH. The HILIC separations can thus be optimized for both dissociated and neutral molecules, which is a merit in terms of retention capacity and selectivity. The zwitterionic phase also enables HILIC separation of inorganic/organic anions and cations within one single run that is a promising approach for the analysis of pharmaceutical salts [2].

Zwitterionic HILIC Stationary Phase



Separation Mechanism



Separation of Cations

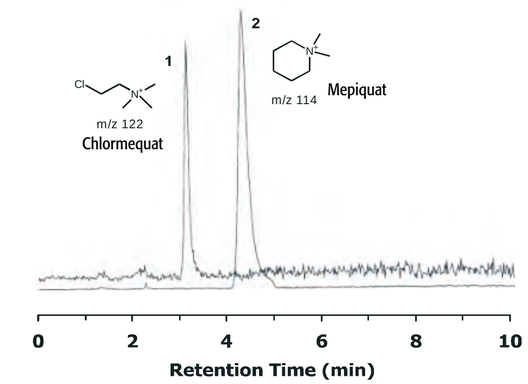


Figure 1. Separation of quaternary amines. Column: ZIC®-HILIC 100 x 2.1 mm, 3.5 µm; Eluent: 80/20 (v/v) acetonitrile/25 mM ammonium acetate, pH 6.8; Flow rate: 0.2 mL/min; Detection: ESI-MS in positive mode, SIM at m/z 114 and 122. By courtesy of Dr.-Ing. Ludmila Havlik, Chemisches Labor, Dr. Wirts + Partner, Hannover, Germany, www.wirts.de.

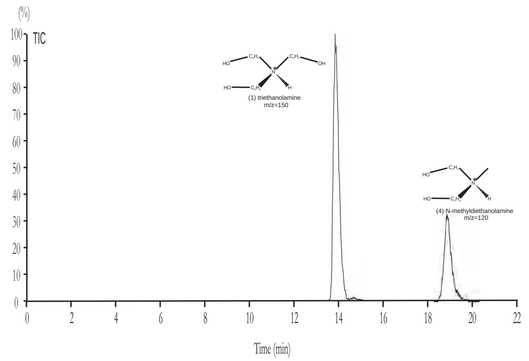


Figure 2. Chromatogram from the separation of two tertiary amines. Column: ZIC®-HILIC 150 x 2.1 mm, 5 µm, 200 Å; Eluent: 70/30 acetonitrile/2.5 mM ammonium acetate buffer, pH 6.8; Flow rate: 120 mL/min and final 10 µL/min split to MS; Detection: ESI-MS in positive mode. By courtsey of Dr. J. Courtois, FOI, Umeå, Sweden.

Separation of Anions

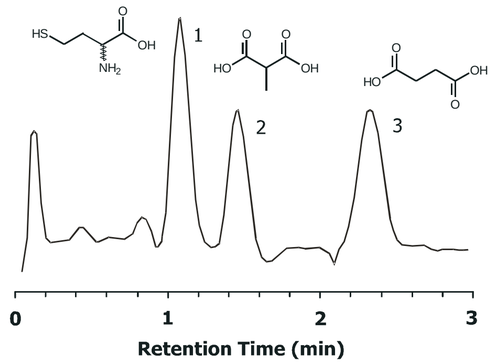


Figure 3. Separation of homocysteine (1), methylmalonic acid (2) and succinic acid. Column: ZIC®-HILIC 50 x 4.6 mm, 5 µm, 200Å; Eluent: 75/25 (v/v) acetonitrile/100 mM NH_4OAc, pH 6.8; Flow rate: 1.0 mL/min; Detection: MS, ESI in positive mode, Capillary voltage 3 kV, Fragmentor 150 V, Mass range 50-200 m/z, Split to MS 100 µL/min; Injection: 5 µL in mobile phase Ref. [3].

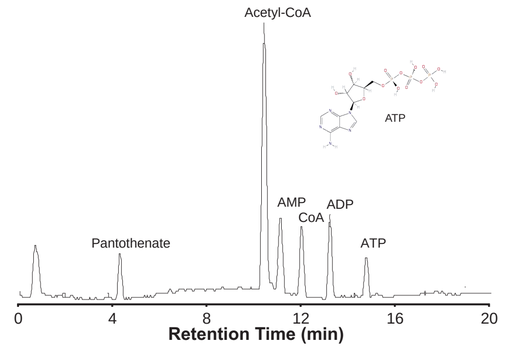


Figure 4. Separation of nucleotides. Column: ZIC®-*p*HILIC 150 x 2.1mm, 5 µm, 200 Å; Eluent: A) ACN, B) 10mM $(NH_4)_2CO_3$+0.2% NH4OH; Gradient: 20-60%B in 15 min, 5 min at 60%B, 15 min equilibration at 20%B; Flow rate: 0.1 mL/min; Detection: FTMS, ESI negative mode, scan 100-1000 m/z; Injection: 1µl (20 pmol).
By courtesy of: T.Pluskal, K.Nagao and M.Yanagida, G0 Cell Unit, Initial Research Project, Okinawa Institute of Science and Technology, Japan

Separation of Zwitterions

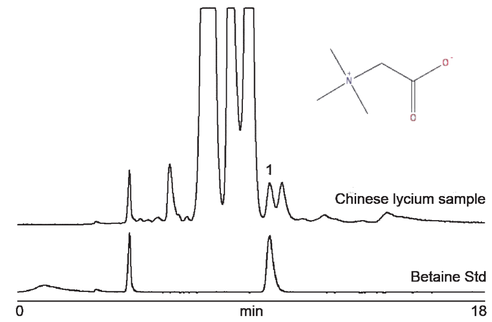


Figure 5. Chromatogram of extracts from Chinese lycium in water. Column: ZIC®-HILIC 250 x 4.6 mm, 5 µm, 200Å; Eluent: 75/25 (v/v) methanol/10mM ammonium acetate, pH 6; Flowrate: 1 mL/min; Detection: PL-ELS 2100 (neb=40°C, evap=30°C, gas=1.6 SLM); Injection: 20 µL. Ref. [4]

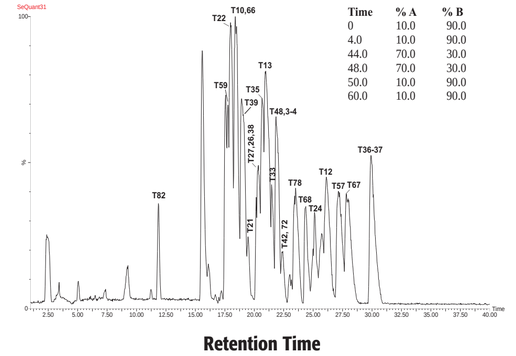


Figure 6. Separation of tryptic digests of bovine serum albumin (BSA). Column: ZIC®-HILIC 150 x 0.3 mm, 5 µm, 200 Å; Gradient elution: Eluent A) H2O 0.25% FA, Eluent B) ACN/0.25% FA, 70/30 (v/v); Flow rate: 5 µL/min; Detection: ESI-MS in positive mode; Sample: BSA tryptic digests, 10 *p*mol/µL.

Simultaneous Separation of Cations and Anions

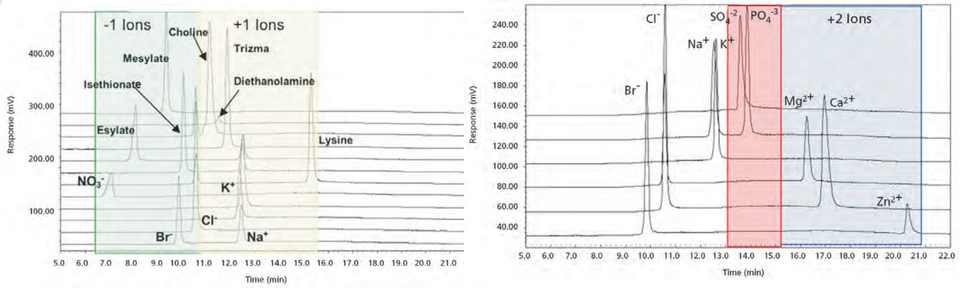


Figure 7. Chromatograms of simultaneous separation cations and anions. Column: ZIC®-HILIC 250 x 4.6 mm, 5 µm, 200Å; Gradient elution: from 85/15(v/v) acetonitrile/100 mM ammonium acetate to 10/90(v/v) acetonitrile/100 mM ammonium acetate in 20 min; Flowrate: 1 mL/min; Detection: Alltech 800 ELSD detector. Ref. [2]

SUMMARY

- Zwitterionic HILIC can separate both organic and inorganic ions
- Zwitterionic HILIC is capable of separating cations, anions and zwitterions using one single column
- The separation of ions is based on mixed-mode separation mechanism, which combines electrostatic interaction and hydrophilic interaction between analytes and zwitterionic stationary phase
- HILIC has excellent compatibility with MS and ELSD detection

For more information or to request a free copy of the booklet *A Practical Guide to HILIC*, please contact info@sequant.com or visit www.sequant.com

References:

[1] P. Hemström, K. Irgum, J. Sep. Sci., 29 (2006) 1784-1821
[2] D.S. Risley and B.W. Pack, LCGC North America, August (2006) 776-785
[3] P. Appelblad et al., LCGC Europe, Suppl. MAR (2005) 47-48
[4] Polymer Laboratories, TB1074: Quantification of Betaine in Chinese Lycium using Hydrophilic Interaction Chromatography (HILIC) and Evaporative Light Scattering Detection (ELSD)